

INDICATORS OF IMMUNOCOMPETENT BLOOD CELLS OF EXPERIMENTAL ANIMALS IMMUNIZED AGAINST PLAGUE ON THE BACKGROUND OF IMMUNOMODULATION

**Pyatidesyatnikova A.B.,
Dubrovina V.I.,
Korytov K.M.,
Kiseleva N.O.,
Balakhonov S.V.**

Irkutsk Antiplague Research Institute
of Siberia and Far East of Rospotrebnadzor
(Trilissera str., 78, Irkutsk 664047,
Russian Federation)

Corresponding author:
Anna B. Pyatidesyatnikova,
e-mail: 50anechka@mail.ru

RESUME

The strategy of specific prophylaxis of plague for epidemic indications accepted in the Russian Federation, while having positive experience, needs to be improved. The solution to one of the problems is the use of vaccines together with adjuvants. The ability of the selenium-containing preparation 974zh to increase the immunogenicity of live plague vaccine was demonstrated.

The aim of the work. To evaluate the subpopulation composition of blood cells in animals immunized by *Y. pestis* EV on the background of immunomodulation.

Materials and methods. In the study we used 100 white mice. Blood served as a material for the study. The phenotype of lymphocytes was determined on a flow cytometer using antibodies to markers CD45, CD44, CD3, CD19, CD4, CD8, CD25, CD62L, I-A/I-E (MHC II).

Results. Evaluation of cellular immunity revealed a statistically significant increase in monocytes on day 7 and a decrease in lymphocytes on day 3 in mice immunized with *Y. pestis* EV at doses of 10^3 CFU and 10^4 CFU in combination with 974zh. When co-injected with *Y. pestis* EV (10^3 CFU) and 974zh, unlike *Y. pestis* EV at different doses, no decrease in $CD3^+CD4^+CD8^+CD25^+$ cells was detected.

Conclusion. Thus, the adjuvant property of 974zh has been established, contributing to an increase in the immunogenic properties of the *Y. pestis* EV vaccine strain, regardless of the dose. The use of *Y. pestis* EV at a dose of 10^3 CFU to reduce the bacterial load is appropriate.

Key words: *Yersinia pestis*, organoselenium compound, lymphocytes, subpopulation composition

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ПОКАЗАТЕЛИ ИММУНОКОМПЕТЕНТНЫХ КЛЕТОК КРОВИ ЭКСПЕРИМЕНТАЛЬНЫХ ЖИВОТНЫХ, ИММУНИЗИРОВАННЫХ ПРОТИВ ЧУМЫ НА ФОНЕ ИММУНОМОДУЛЯЦИИ

**Пятидесятникова А.Б.,
Дубровина В.И.,
Корытов К.М.,
Киселева Н.О.,
Балахонов С.В.**

ФКУЗ Иркутский научно-исследовательский противочумный институт Роспотребнадзора (664047, г. Иркутск, ул. Трилиссера, 78, Россия)

Автор, ответственный за переписку:
**Пятидесятникова Анна
Борисовна,**
e-mail: 50anechka@mail.ru

РЕЗЮМЕ

Принятая в Российской Федерации стратегия проведения специфической профилактики чумы по эпидемическим показаниям, несмотря на положительный опыт, нуждается в совершенствовании. Решением одной из проблем является применение вакцин совместно с адъювантами. Продемонстрирована способность селенсодержащего препарата 974zh повышать иммуногенность живой чумной сухой вакцины.

Цель работы. Оценка субпопуляционного состава клеток крови животных, иммунизированных *Y. pestis* EV на фоне иммуномодуляции.

Материалы и методы. Биомоделями служили 100 белых мышей, материалом для исследования – кровь. Фенотип лимфоцитов определяли на проточном цитофлуориметре с использованием антител к маркерам CD45, CD44, CD3, CD19, CD4, CD8, CD25, CD62L, I-A/I-E (MHC II).

Результаты. При оценке клеточного иммунитета выявлено статистически значимое увеличение моноцитов на 7 сутки и снижение лимфоцитов на 3 сутки у мышей, иммунизированных *Y. pestis* EV дозами 10^3 КОЕ и 10^4 КОЕ в сочетании с 974zh. Отмечено повышение CD3⁺CD4⁺CD8⁻CD25⁺ клеток в опытной группе животных при сочетанном введении *Y. pestis* EV (10^3 КОЕ) и 974zh.

Заключение. Таким образом, установлено адъювантное свойство препарата 974zh, способствующее повышению иммуногенных свойств вакцинного штамма *Y. pestis* EV не зависимо от дозы. Поэтому для уменьшения бактериальной нагрузки целесообразно использовать *Y. pestis* EV в дозе 10^3 КОЕ.

Ключевые слова: *Yersinia pestis*, селеноорганическое соединение, лимфоциты, субпопуляционный состав

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INTRODUCTION

Plague is an infectious disease caused by the *Yersinia pestis* bacteria. Although the infection is rare in the modern world, it remains a public health concern. Vaccination plays a significant role in controlling the spread of plague. Plague vaccination is administered according to the preventive vaccination schedule approved by the Ministry of Health of the Russian Federation, Order No. 1122n dated December 6, 2021. This vaccination is recommended for individuals temporarily or permanently residing in areas where plague outbreaks occur, as well as for those working with live plague pathogens. A licensed live plague vaccine (LPV) is used in the Russian Federation for specific plague prevention [1]. Long-term experience in the immunological monitoring of individuals vaccinated against plague living in the areas of three natural plague foci (Caspian sandy, Gorno-Altai highlands, and Tuva mountains) has demonstrated the efficacy of using live subunit vaccines [2]. At the same time, most vaccine preparations based on weakened; repeatedly passaged microorganisms can be reactogenic and cause allergic reactions. In contrast, subunit vaccines are safer but have lower immunogenicity [3]. To address the issues of vaccine reactogenicity and low subunit vaccine immunogenicity, adjuvants have been used [4]. The search for adjuvants that can activate innate immune responses, reduce antigen load, trigger and enhance adaptive immune response remains a significant and promising area of research [5].

We have previously demonstrated the immunomodulatory properties of the experimental synthetic organoselenium compound 2,6-dipyridinium-9-selenabicyclo[3.3.1]nonane dibromide (974zh) [6], which increases the immunogenicity of the *Y. pestis* EV NIEG vaccine strain, even when the immunizing dose is reduced, and also reduces allergic reactions and enhances the immune response to the vaccine [7, 8].

Considering the potential of drug 974zh to enhance the immunogenicity of the *Y. pestis* EV NIEG vaccine strain, a study of the cellular composition of blood lymphocytes utilizing flow cytometry would allow us to determine the characteristics of the impact of this medication on the cellular aspect of immunity [9].

THE AIM OF THE STUDY

To assess the subpopulation composition of blood cells in immunized white mice with *Y. pestis* EV NIEG in the context of immunomodulation using the drug 974zh.

MATERIALS AND METHODS

The work with the *Y. pestis* EV vaccine strain and experimental animals was conducted in accordance with the SanPiN 3.3686-21 «Sanitary and Epidemiological

Requirements for the Prevention of Infectious Diseases», as well as the requirements of Directive 2010/63/EU on the protection of animals used for scientific purposes, and the «Rules of Good Laboratory Practice», approved by Order No. 199n of the Ministry of Health dated April 1, 2016. The study was approved by the local ethics committee of the institution (protocol No. 7, dated November 15, 2021).

The study focused on the synthetic organoselenium compound 1,5-diparidinonium-9-selenobicyclo [3.3.1]nonane dibromide (1-[6-(1-pyridinium)-9-selenabicyclo[3.3.1]non-2-yl]pyridinium dibromide (974zh). This compound was synthesized in the laboratory of organochalcogen compounds at the A.E. Favorsky Institute of Chemistry of the Siberian Branch of the Russian Academy of Sciences, and it was used in conjunction with the *Y. pestis* vaccine strain EV line NIEG.

The study involved 100 outbred, white mice of both sexes, weighing 18–22 g, obtained from the Irkutsk Anti-Plague Research Institute (RD 42-26-3...3738, State Research Center for Virology and Biotechnology «Vector», Novosibirsk). The animals were divided into five groups, each consisting of 20 individuals. The first group (group I) received a subcutaneous injection of 0.5 ml of *Y. pestis* EV at a dose of 10^4 CFU. The second group (group II) received a combination of *Y. pestis* EV (10^4 CFU) and 974zh, at a dose of 2.5 mg/kg. The third group (group III) received *Y. pestis* EV at a dose of 10^3 CFU. Group IV received a combination of both *Y. pestis* EV (10^3 CFU) and 974zh (2.5 mg/kg). Animals in the control group received an isotonic sodium chloride solution pH 7.2 (0.5 ml). Research and data collection were conducted on days 3, 7, 14, and 21. Heparinized blood samples were used for the study.

To determine the phenotype of lymphocytes, a panel of monoclonal antibodies (mAbs) from Becton Dickinson (USA), specific for the markers CD45, CD44, CD3, CD19, CD4, CD8, CD25, CD62L, I-A/I-E (MHC II), were utilized.

A blood cell subpopulation analysis was conducted using a BD FACSCanto™ II flow cytometer and BD Diva 6.0 software. Statistical analysis of the obtained data was performed using nonparametric methods and Statistica 6.0 software. The results are presented as median values and interquartile ranges (IQR). Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

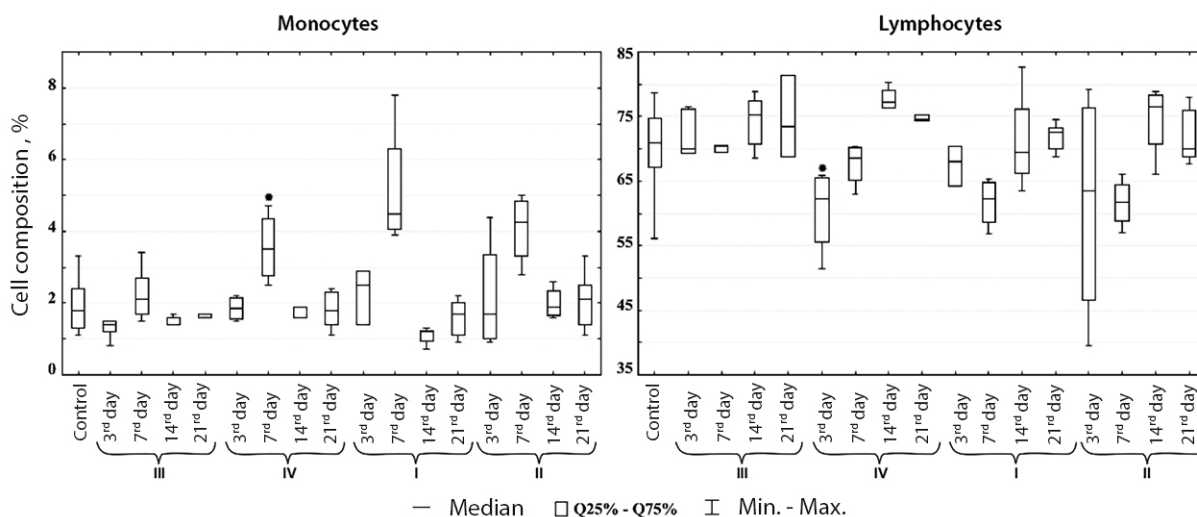
In group IV of the experimental animals, a statistically significant increase ($p < 0.05$) in the percentage of monocytes was observed on day 7, by 1.7-fold (3.5 (2.8–4.4)%) compared to group III (2.1 (1.7–2.7)%) and a decrease in lymphocytes by 1.2-fold (62.3 (55.5–65.4)%) on day 3. Groups I and II showed a similar change in these indicators on day 7, which was not statistically significantly different from the values in group IV (Fig. 1).

During the experiment, an increase in the number of T-lymphocytes and a decrease in B-lymphocytes were observed in animals from experimental groups I and III on the third day, with no statistically significant differences between the groups.

It was demonstrated that the expression levels of CD44 and CD62L molecules on the surface of T-lymphocytes (naive $CD3^+CD44^-CD62L^+$) in group IV on day 21 were statistically significantly lower by 1.2-fold ($p < 0.05$)

compared to those in group II of animals. The proportion of naive T-cells was (78.0 (64.7–78.3)%) in experimental group IV and (83.4 (80.5–83.7)%) in group II (Fig. 2).

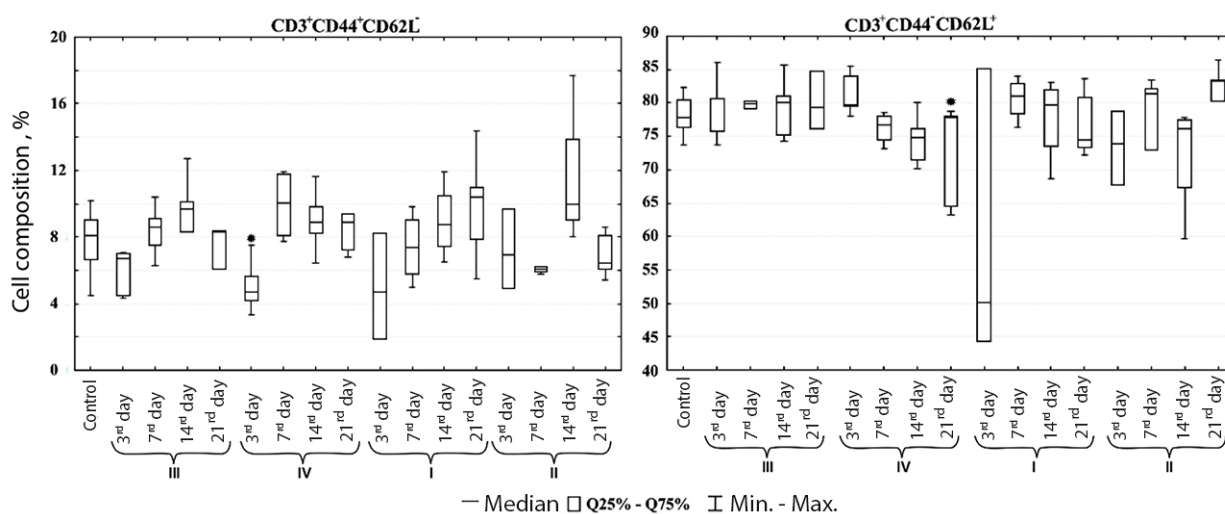
A statistically significant decrease of $CD3^+CD4^+CD8^-CD44^+CD62L^-$ cells by 1.7-fold was observed on day 3 in group IV, compared to the control group (6.5 (4.6–6.8)%). A statistically significant increase in the number of these cells was detected in the blood of animals from group II on day 14 (8.3 (7.5–10.5)%),



Note. I – *Y. pestis* EV at a dose of 10^4 CFU, II – *Y. pestis* EV at a dose of 10^4 CFU in combination with 974zh, III – *Y. pestis* EV at a dose of 10^3 CFU, and IV – *Y. pestis* EV at a dose of 10^3 CFU in combination with 974zh; * – $p < 0.05$ compared to group III.

FIG. 1.

Dynamics of the content of monocytes and lymphocytes in the blood of experimental animals at different stages of the experiment with immunization with *Y. pestis* EV in different doses and with combined administration with 974zh, Me (Q25%–Q75%)



Note. I – *Y. pestis* EV at a dose of 10^4 CFU, II – *Y. pestis* EV at a dose of 10^4 CFU in combination with 974zh, III – *Y. pestis* EV at a dose of 10^3 CFU, and IV – *Y. pestis* EV at a dose of 10^3 CFU in combination with 974zh; * – $p < 0.05$ compared to group II.

FIG. 2.

Expression of adhesion molecules CD44 and CD62L on T-lymphocytes, Me (Q25%–Q75%)

which is 1.3-fold higher than the values in the control group ($p < 0.05$). No changes were observed in the CD8⁺ population (naive T-lymphocytes, memory T-cells).

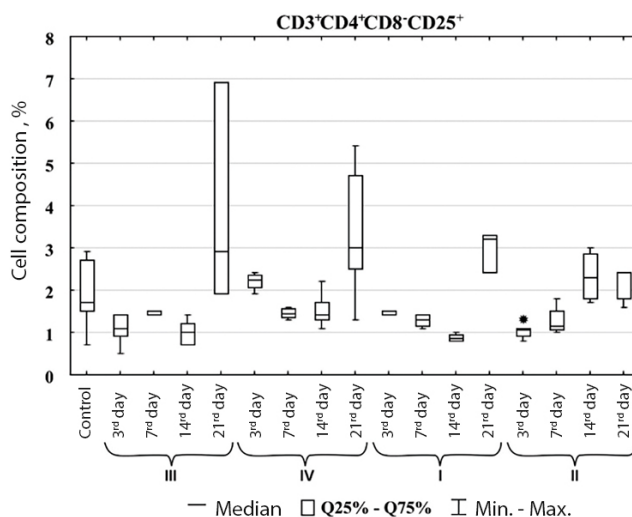
In experimental groups I, II, and III, during the determination of the dynamics of circulating activated helper T-lymphocytes in the blood of experimental animals, a statistically significant reduction in CD3⁺CD4⁺CD8⁺CD25⁺ cells was observed on days 3, 7, and 14. A comparative analysis of these parameters between groups II and IV revealed that on the third day of the study, the content of these cells in animals of group II was 2.1-fold lower than that in animals of group IV ($p < 0.05$). This difference is likely associated with a higher antigen load in group II (Fig. 3).

The number of activated T-helpers (CD3⁺CD4⁺CD8⁺CD44⁺CD62L⁺CD25⁺ – and CD3⁺CD4⁺CD8⁺CD44⁺CD62L⁺CD25⁺ cells) in the blood of white mice from all experimental groups on day 3 was significantly lower than the control values. On day 14 of the immunogenic process, regardless of the dose of *Y. pestis* EV administered, there was a tendency towards an increased number of activated naive T-helpers in experimental animals from groups I and III (Fig. 4).

It is worth noting that in group II of animals on day 14 and group IV on day 7, there was an increase in the number of memory helper T-cells expressing CD25⁺ (Fig. 4).

CONCLUSION

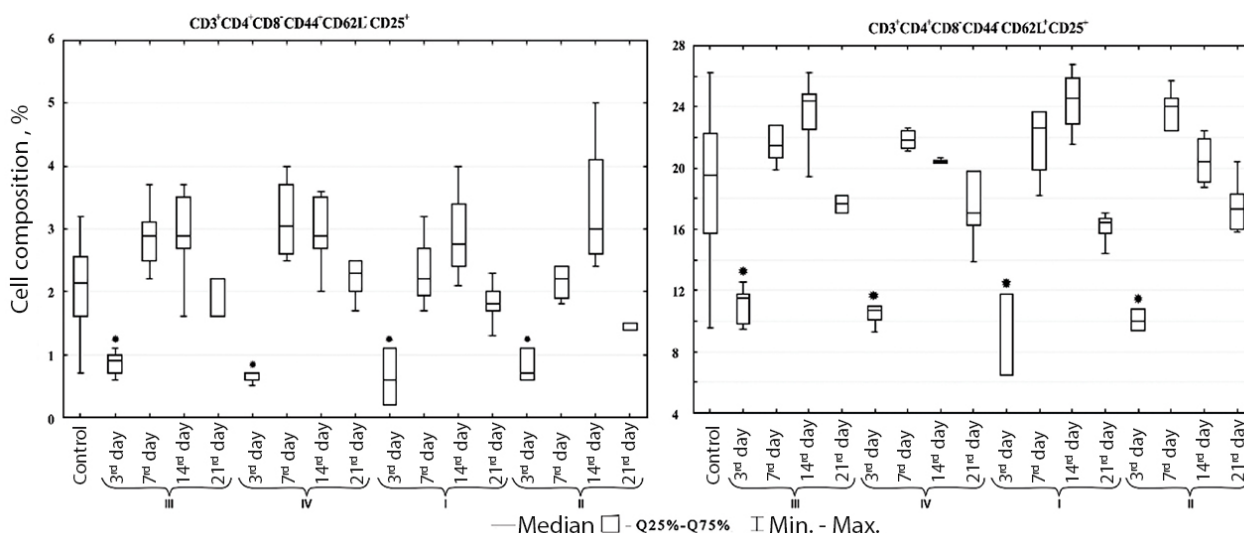
The study has demonstrated the immunomodulatory effects of the organoselenium compound 974zh on innate immune cell activation, even with a reduced



Note. I – *Y. pestis* EV at a dose of 10⁴ CFU, II – *Y. pestis* EV at a dose of 10⁴ CFU in combination with 974zh, III – *Y. pestis* EV at a dose of 10³ CFU, and IV – *Y. pestis* EV at a dose of 10³ CFU in combination with 974zh; * – $p < 0.05$ compared to group IV.

FIG. 3. Dynamics of the content of circulating CD25⁺-activated helper T-lymphocytes, Me (Q25%–Q75%)

immunizing dose of the *Y. pestis* EV vaccine strain. This compound has been shown to influence the composition of blood cell populations in experimental animals, regardless of the dose of vaccine used (10³ or 10⁴ CFU). Importantly, there was no decrease in the number of CD3⁺CD4⁺CD8⁺CD25⁺ cells when 974zh was combined with *Y. pestis* at a dose of 10³ CFU.



Note. I – *Y. pestis* EV at a dose of 10⁴ CFU, II – *Y. pestis* EV at a dose of 10⁴ CFU in combination with 974zh, III – *Y. pestis* EV at a dose of 10³ CFU, and IV – *Y. pestis* EV at a dose of 10³ CFU in combination with 974zh; * – $p < 0.05$ compared to control.

FIG. 4. Content of activated CD25⁺ T helper lymphocytes in the blood of white mice Me (Q25%–Q75%)

Therefore, the adjuvant effect of 974zh has been demonstrated, enhancing the immunogenicity of the *Y. pestis* EV vaccine strain, regardless of dose. Therefore, in order to reduce the bacterial burden, it is recommended to use *Y. pestis* EV at a dose of 10^3 CFU.

Based on the available information regarding the effect of drug 974zh on the immunogenic and protective activity of the *Yersinia pestis* EV NIIEG vaccine strain, as well as our findings, it is evident that further research is needed to pathogenetically substantiate its impact on the cells of the macroorganism's immune system.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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Information about the authors

Anna B. Pyatidesyatnikova – Junior Research of Pathophysiological Laboratory of Irkutsk Antiplague Research Institute of Siberia and Far East of Rospotrebnadzor; e-mail: adm@chumin.irkutsk.ru, <https://orcid.org/0000-0002-6381-4517>

Valentina I. Dubrovina – Dr. Sc. (Biol.), Head of Pathophysiological Laboratory of Irkutsk Antiplague Research Institute of Siberia and Far East of Rospotrebnadzor; e-mail: dubrovinavalya@mail.ru, <https://orcid.org/0000-0001-8561-6207>

Konstantin M. Korytov – Research Officer, Pathophysiological Laboratory, Irkutsk Antiplague Research Institute of Siberia and Far East of Rospotrebnadzor; e-mail: konstmikhkor@yandex.ru, <https://orcid.org/0000-0003-1137-6049>

Natalya O. Kiseleva – Junior Research of Pathophysiological Laboratory of Irkutsk Antiplague Research Institute of Siberia and Far East of Rospotrebnadzor; e-mail: adm@chumin.irkutsk.ru, <https://orcid.org/0000-0001-6678-2998>

Sergey V. Balakhonov – Dr. Sc. (Med.), Professor, Director of Irkutsk Antiplague Research Institute of Siberia and Far East of Rospotrebnadzor; e-mail: adm@chumin.irkutsk.ru, <https://orcid.org/0000-0003-4201-5828>